Gas Chromatography and Mass Spectroscopy

Baseline Liquid Culture Preparations:

- A 10ml liquid culture of each ATF1 construct is prepared beforehand from plate based colonies, with appropriate antibiotic. Allow at least 12 hours for proliferation of cells in a warm environment. When preparing, reserve time for the gas chromatography machine as necessary, chromatographs from cells will contain significant amounts of noise, nullifying any viable results.

Example:

ATF1 with Arsenic induced Promoter biobrick, (as well as RBS and DT)
ATF1 with ATF1 with Constitutive Promoter biobrick, (as well as RBS and DT)

Experimental Procedure

A. We decided on what experimental conditions we would test for, our example is provided below, all tubes included 2ml LB and 5ul of antibiotic appropriate for backbone:

1. Constitutive Promoter E.Coli (Negative Control)
2. Constitutive Promoter E.Coli + 5mM Isoamyl Alcohol (Theoretical Maximum Yield))
3. Arsenic Promoter E.Coli (Negative Control)
4. Arsenic Promoter E.Coli + 5mM Isoamyl Alcohol (Negative Control for presence of Arsenic)
5. Arsenic Promoter E.Coli + 100uM Arsenite (Negative Control for presence of Isoamyl Alcohol)
6. Arsenic Promoter E.Coli + 5mM Isoamyl Alcohol + 0.5uM Arsenite
7. Arsenic Promoter E.Coli + 5mM Isoamyl Alcohol+ 5uM Arsenite
8. Arsenic Promoter E.Coli + 5mM Isoamyl Alcohol+ 10uM Arsenite
9. Arsenic Promoter E.Coli + 5mM Isoamyl Alcohol+ 50uM Arsenite
10. Arsenic Promoter E.Coli + 5mM Isoamyl Alcohol+ 100uM Arsenite
11. 5mM Isoamyl Alcohol + 5mM Isoamyl Acetate
12. 2x LB dilution of #11
13. 10x LB dilution of #11
14. 25x LB dilution of #11
15. 50x LB dilution of #11

B. The total liquid culture is diluted with LB to an O/D of 0.20, this creates an equal distribution of cells throughout the liquid culture.
C. For our experiment, we prepared 35 labeled and autoclaved tubes. One for each of our planned readings. For accuracy, we performed each of the 10 experimental trials over three tubes.
D. The materials were injected into each tube, and parafilm was placed over the tube for an airtight seal
E. These tubes were placed into a warm shaker for 15 hours overnight.
F. Once taken out, 800ul of liquid was taken out and injected into an eppie, these eppies were centrifuged at 13,000 RPM for 1 minute. This step is to separate out E.Coli cells from the solution.
G. The liquid from each eppie is taken out, and then injected into a THOMPSON INTRUMENT COMPANY "PTFE Standard Filter Vial 0.45μM", part **#35540-200.**
H. These vials placed taken to the gas chromatograph, and were run through the machine using these parameters:

GC/MS Method Control Parameters

The Full Readout is available on the next four pages, but included directly below are the important variable settings that the machine must be set to.

Oven Program: On, 50C for 2 min, then 20C/min to 280C for 2 minutes. Runtime = 15.5 Minutes

Injection Volume: 2ul

Mode: Split

Split ratio: 50:1

Thermal Aux 2 {MSD Transfer Line}
-> Heater: On
-> Temperature Program: 280C for 0min

MS Information

Solvent Delay: 2.50min
Low Mass: 30.0
High Mass: 400.0

MS Source: 230C
MS Quad: 150C

METHOD CONTROL PARAMETERS

Method Information for: D:\MSDCHEM\1\METHODS\FARNY_IGEM_ISOAMYLACETATE_070914_1.M

Method Sections To Run:

(X) Save Copy of Method With Data
( ) Instrument Control Pre-Run Cmd/Macro =
( ) Data Analysis Pre-Run Cmd/Macro =
(X) Data Acquisition
(X) Data Analysis
( ) Instrument Control Post-Run Cmd/Macro =
( ) Data Analysis Post-Run Cmd/Macro =

Method Comments:
FARNY_IGEM_ISOAMYLACETATE_070914_1.M is the first attempt at a method for analysis of isoamyl acetate (product) and isoamyl alcohol (reactant), samples are growth media from e. coli cultures, containing LB media and water, analysis is performed with Natalie FARNY as part of IGEM,
APB

END OF METHOD CONTROL PARAMETERS

INSTRUMENT CONTROL PARAMETERS: GC MSD

D:\MSDCHEM\1\METHODS\FARNY_IGEM_ISOAMYLACETATE_070914_1.M
Wed Jul 09 15:57:42 2014

Control Information

Sample Inlet	:	GC
Injection Source	:	GC ALS
Mass Spectrometer	:	Enabled

No Sample Prep method has been assigned to this method.

Oven	
Equilibration Time	1 min
Max Temperature	325 degrees C
Slow Fan	Disabled
Oven Program	On
50 °C for 2 min	
then 20 °C/min to 280 °C for 2 min	
Run Time	15.5 min
1 min (Post Run)	50 °C

Front Injector	
Syringe Size	10 µL
Injection Volume	2 µL
Solvent A Washes (PreInj)	4
Solvent A Washes (PostInj)	4
Solvent A Volume	8 µL
Solvent B Washes (PreInj)	4
Solvent B Washes (PostInj)	4
Solvent B Volume	8 µL
Sample Washes	2
Sample Wash Volume	2 µL
Sample Pumps	3
Dwell Time (PreInj)	0 min
Dwell Time (PostInj)	0 min
Solvent Wash Draw Speed	300 µL/min
Solvent Wash Dispense Speed	6000 µL/min
Sample Wash Draw Speed	300 µL/min
Sample Wash Dispense Speed	6000 µL/min
Injection Dispense Speed	6000 µL/min
Viscosity Delay	0 sec
Sample Depth	2 mm
Injection Type	Standard
L1 Airgap	0.2 µL

Sample Overlap
Sample overlap is not enabled

Front SS Inlet He		
Mode		Split
Heater	On	280 °C
Pressure	On	7.6522 psi
Total Flow	On	54 mL/min
Septum Purge Flow	On	3 mL/min
Gas Saver	On	20 mL/min After 3 min
Split Ratio		50 :1
Split Flow		50 mL/min

Thermal Aux 2 (MSD Transfer Line)
Heater On
Temperature Program On
    280 °C for 0 min
Run Time 15.5 min

Column #1
Agilent 19091S-433UI: 2485.66698
HP-5MS Ultra Inert 5% Phenyl M
325 °C: 30 m x 250 µm x 0.25 µm
In: Front SS Inlet He
Out: Vacuum

(Initial) 50 °C
Pressure 7.6522 psi
Flow 1 mL/min
Average Velocity 36.445 cm/sec
Holdup Time 1.3719 min
Flow Program Off
    1 mL/min for 0 min
Run Time 15.5 min
1 min (Post Run) 1.2 mL/min

Signals
Test Plot Save Off
50 Hz
Test Plot Save Off
50 Hz
Test Plot Save Off
50 Hz
Test Plot Save Off
50 Hz

MS ACQUISITION PARAMETERS

General Information
------- -----------

Tune File : atune.u
Acquistion Mode : Scan

MS Information
-- -----------

Solvent Delay : 2.50 min

EMV Mode : Relative
Relative Voltage : 0
Resulting EM Voltage : 1729

[Scan Parameters]

Low Mass : 30.0
High Mass : 400.0
Threshold : 500
Sample # : 2 A/D Samples 4
Plot 2 low mass : 30.0
Plot 2 high mass : 400.0

[MSZones]

MS Source : 230 C maximum 250 C
MS Quad : 150 C maximum 200 C

END OF MS ACQUISITION PARAMETERS

TUNE PARAMETERS for SN: US10287505

Trace Ion Detection is OFF.

EMISSION	:	34.610
ENERGY	:	69.922
REPELLER	:	34.814
IONFOCUS	:	90.157
ENTRANCE_LE	:	25.500
EMVOLTS	:	1729.412
		Actual EMV : 1729.41
		GAIN FACTOR : 2.88
AMUGAIN	:	1863.000
AMUOFFSET	:	120.938
FILAMENT	:	2.000
DCPOLARITY	:	0.000
ENTLENSOFFS	:	17.820
MASSGAIN	:	-708.000
MASSOFFSET	:	-39.000

END OF TUNE PARAMETERS

END OF INSTRUMENT CONTROL PARAMETERS